

# THE TREATMENT OF DERMAL TUMORS, WARTS, AND VIRAL INFECTIONS OF THE RESPIRATORY TRACT IN HUMANS USING HEAT-KILLED *P. ACNES*

## Field of the Invention

The present invention relates to methods to treat viral infections, dermal tumors, and warts in humans using heat-killed bacterial compositions. Specifically, it relates to the subcutaneous or intralesional administration of heat-killed *Propionibacterium acnes* (*P. acnes*), to treat dermal tumors and warts, and to the oral administration of heat-killed *P. acnes* to treat virus induced infections of the respiratory tract in humans.

## Background of the Invention

The maintenance of a healthy and competent immune system is a prerequisite for resistance to and elimination of infectious and neoplastic diseases. Bacteria and their derivatives were among the first substances to be recognized as immunostimulators and are used as adjuvants in vaccines to boost the humoral immune response (*e.g.*, complete Freund's adjuvant). Bacteria have also been used as non-specific enhancers of the immune system to increase resistance and rejection of cancers, parasites, and infectious organisms.

Gram positive, whole-cell bacteria such as *Propionibacterium acnes*, *Propionibacterium avidum*, *Propionibacterium lymphophilum*, *Propionibacterium granulosum*, *Corynebacterium parvum* and *Arachnia propionica*, when inactivated have been shown to be potent non-specific immune stimulants in animals and humans. Specifically *Propionibacterium acnes* (*P. acnes*) has been shown to stimulate antineoplastic activity, adjuvant activity, antiviral activity, antibacterial activity, and to stimulate hematopoiesis.

Preparations of *P. Acnes* have been shown to act as non-specific stimulators of immunogenic responsiveness in vivo. *P. Acnes* is known to act by stimulating macrophages and neutrophils, initiating endogenous production of lymphokines (including IL-2 and various interferons), and enhancing killer cell activity. The intranasal inoculation of mice with *P. acnes* has been shown to activate pulmonary macrophages (Jackson RA, et al., *J Leukoc. Biol.*, 40(5):575-87, 1986). At the cellular level, *P. acnes* acts upon monocytes and lymphocytes and improves the functional interaction between these cells (M.T. Scott, *Cell Immunol.*, 17:141, 1975).

1           *P. acnes* also functions as an immune adjuvant to weakly antigenic substances. These  
2 properties, while not completely understood, play an important role in regulation of the immune  
3 response. One mode of the interaction of inactivated *P. acnes* with the immune system is through its  
4 stimulation of the reticuloendothelial system (RES), *i.e.* liver, spleen, lymph nodes, lungs, and bone  
5 marrow (C. Adlam, and M.T. Scott, *J. Med Microbiol*, 6:621 (1973), N.H. McBride *et al.*, *Cell*  
6 *Immunol.*, 7:290 (1973)).

7           This activity elicits enhanced resistance to bacterial and viral infections, and also to certain  
8 tumors. The mode of action appears to be the activation of macrophages followed by the recruitment  
9 of lymphocytes. The particulate nature of *P. acnes* appears important for macrophage activation.  
10 Unlike some synthetic biological response modifiers (BRM's), bacteria *in vivo* are fully degraded and  
11 catabolized in the body without the formation and excretion of toxic metabolites or retention of  
12 residues. This has obvious therapeutic advantages for *P. acnes*. and contributes to the therapeutic and  
13 prophylactic use of *P. acnes* against infectious diseases.

14           In animals, stimulation of the immune system results in short term protection against infection  
15 with certain viruses and bacteria. Used therapeutically in animals with chronic skin and respiratory  
16 disease, *P. acnes* shortens the course of the disease.

17           The anti-tumor activity of *P. acnes* has been studied in mice and other animals. Tumor cells  
18 injected into Balb/c mice together with heat-killed *P. acnes* cells were rendered nontumorigenic  
19 (Murano EA, *et al*, *Cancer Immunol Immunother*, 29(1):7-16, 1989). The preventive effect of *P.*  
20 *acnes* on metastasis in mice rendered tolerant to tumor-associated transplantation antigens (TATA) has  
21 been detailed (Fujiwara H, *et al* , *Gann*, 71(5):692-8, 1980). Heat-killed suspensions of several *P.*  
22 *acnes* strains were prepared and studied for their protective activity against viral infections in mice and  
23 for their immunomodulating properties (Zgorniak-Nowosielska I, *et al*, *Arch Immunol Ther Exp*  
24 *(Warsz)*, 37(3-4):431-42, 1989).

25           There has been considerable data collected on the use of *P. Acnes* in domestic animals. In a  
26 randomized study conducted for the treatment of equine respiratory disease (ERDC), complete  
27 recovery within a 14 day period was observed in horses treated intravenously with *P. acnes* (D. R.  
28 Evans *et al.*, *Equine Practice*, 10:17, 1988; C.D. Vail *et al.*, *Vet. Review*, Nov/Dec: 399, 1990).

1 Additionally, inactivated *P. acnes* has also been shown to be a biological response modifier for  
2 treatment of non-specific respiratory diseases in horses where upon administration of *P. acnes* it was  
3 shown that CD4+ lymphocyte expression and lymphokine activated killer cell (LAK) activity increased  
4 (Flaminio MJ, *et al*, *Vet Immunol Immunopathol*, 63(4):303-15, 1998).

5 In a randomized, double blinded, placebo controlled study, dogs with a significant skin disease  
6 (chronic recurrent pyoderma) were treated with antibiotics plus *P. acnes* with significant improvement  
7 or complete remission of the lesions (A. Becker *et al.*, *J. Vet Intern. Med.* 13:26 (1989)).

8 *P. acnes* has been extensively used as a veterinary therapeutic in cattle with papilloma (warts)  
9 where the warts had been intralesionally injected with *P. acnes* (H. Hall *et al.*, *Therapeutic*  
10 *Immunology*, 1:319, 1994). While, lesions in the control group which were injected with saline  
11 showed no regressions at the end of 16 weeks, 100% of the injected lesions in the treatment group had  
12 completely regressed at the end of 16 weeks.

13 Use of *P. acnes* in humans has, in general been limited to treatment of neoplastic diseases and  
14 pleural effusions with some limited success. Additionally, *P. acnes* has been administered orally in the  
15 rations of food production animals to promote better health through cell-mediated immunity and weight  
16 gain (U.S. Patent Application Serial No. 08/912,026). It has been used experimentally in people to  
17 treat various cancers, plural effusion and chronic obstructive pulmonary disease. It has been used  
18 experimentally as an adjuvant with vaccines.

19 Based on these findings, a veterinary preparation of *P. acnes* was used as an injectable  
20 therapeutic agent against plantar warts caused by the human papilloma virus. However, significant pain  
21 upon injection was observed caused due to the alcohol content of the preparation. Thus, a preparation  
22 of *P. acnes* is needed that causes the regression of warts and dermal tumors in humans, but which may  
23 be administered without undue pain or harm to the patient. Additionally, this preparation must be  
24 administered via a route that allows regression of the warts while minimizing pain to the patient .

25 Although *P. acnes* has been used to treat respiratory diseases in horses and cattle, the oral  
26 administration of *P. acnes* with efficacy in humans has not been previously demonstrated. There is a

1 need for a *P. acnes* preparation that can be safely administered to humans for the treatment of viral  
2 infections of the respiratory tract.

3 *P. acnes* preparations have been administered primarily through intravenous, intraperitoneal, or  
4 intrathoracic routes. However, they may also be administered orally, subcutaneously, or intralesionally  
5 depending on the type of infection and the determined dosage. *P. acnes* has been used at higher dose  
6 levels in experimental animals to study the release of nitric oxide by cells or the liver and other body  
7 tissues, and has been combined with vaccines as an adjuvant for subcutaneous or intramuscular  
8 injection. Ethanol-saline suspended preparations of heat-killed *P. acnes* for veterinary use in treating  
9 pyoderma, a bacterial infection in dogs, and respiratory infections in horses have been used. However,  
10 these preparations had to be administered intravenously in order to be efficacious. In another case, a  
11 feed additive consisting of dried *P. acnes* mixed with feed rations was given to baby pigs which  
12 subsequently exhibited decreased mortality, increased weight gain and feed conversion. However,  
13 optimization of the route of administration for the treatment of dermal warts, tumors, and viral infections  
14 of the respiratory tract in humans has not hitherto been conducted.

15 In order to efficaciously administer the *P. acnes* preparation, an optimal mode of inactivation of  
16 the *P. acnes* preparation is also needed. Although, suspending the *P. acnes* in an ethanol-saline  
17 suspension causes inactivation of *P. acnes*, the presence of ethanol causes discomfort in humans. Thus,  
18 there is a need to safely and adequately inactivate the *P. acnes* without any undue loss in activity.  
19 Heat-killing is an efficacious method of inactivating *P. acnes*. However, there is a need to develop a  
20 method of heat-killing that adequately inactivate the *P. acnes* while maintaining desired levels of activity.

## 21 **Summary of the Invention**

22 This is an invention to induce regression of a virally induced dermal tumor, especially plantar  
23 warts for which painful surgical removal or chemical burning are the most common methods of removal.  
24 These alternate methods cause severe pain and limit mobility to a majority of patients receiving these  
25 treatments. It is also an invention to treat and hasten recovery from virally induced infection of the  
26 respiratory tract using autoclaved *P. acnes* through a novel route of administration, previously not  
27 demonstrated in man, that of oral administration.

1 This invention also relates to the preparation of an alcohol-free, terminally sterilized saline-  
2 suspended *P. acnes* product that causes the regression of dermal tumors, and plantar warts in humans.  
3 Terminal sterilization may be conducted through the process of autoclaving. In another embodiment of  
4 the product, an anesthetic such as lidocaine is added to the *P. acnes* product. The invention also  
5 relates to a novel intralesional administration of the *P. acnes* product into plantar warts, or other warts  
6 caused by the human papilloma virus causing regression of such warts, and the subcutaneous  
7 administration of the *P. acnes* product resulting in a systemic regression of warts.

### 8 Detailed Description of the Invention

9 This invention relates to the preparation, administration, and use of an inactivated bacterial  
10 product to induce regression of virally induced dermal tumors and warts, and to effectively treat virally  
11 induced infections of the respiratory tract. The warts may be plantar, genital, or surface warts  
12 anywhere on the skin or mucosal surface of the body, or those caused by the human papilloma virus.

13 The bacteria used for practicing the invention may be selected from the group consisting of  
14 *Propionibacterium acnes*, *Propionibacterium avidum*, *Propionibacterium lymphophilum*,  
15 *Propionibacterium granulosum*, *Cornynebacterium parvum* or *Arachnia propionica*. Preferably,  
16 the bacteria used for practicing the invention are selected from the *Propionibacterium* family. Most  
17 preferably, the bacteria used for practicing the invention is *Propionibacterium acnes* (*P. acnes*).  
18 Thus, *P. acnes* will be the bacterium referred to throughout the description, although any of the  
19 bacterial species claimed can be substituted. However, the statements contained in this description  
20 should apply to each of the bacteria claimed unless otherwise indicated, since all of the claimed bacteria  
21 are expected to have the same results due to their taxonomic similarity. Although it is now recognized  
22 that *Cornynebacterium parvum* (*C. parvum*) is thought to be synonymous with *P. acnes*, it has been  
23 included in the list due to the use of the name that still exists in the art.

24 In the present invention, a method for preparing a saline suspension of heat-killed *P. acnes* with  
25 demonstration of potency through a laboratory animal challenge model is disclosed. It has been  
26 determined that heat-killing, which usually destroys or alters the antigens needed to stimulate the  
27 immune responses, does not destroy the potency of the autoclaved *P. acnes* product. Furthermore, as

1 shown in laboratory animal potency tests, the addition of an anesthetic such as lidocaine to the  
2 autoclaved *P. acnes* product does not destroy the potency of the *P. acnes* product.

3 *P. acnes* is known to be commercially available in forms such as an injectable solution (e.g.,  
4 ImmunoRegulin<sup>®</sup> or EqStim<sup>®</sup> by Neogen Corp. (Lansing, MI)), but it may also be isolated and cultured  
5 by known, standard bacterial procedures or obtained from national culture collections. The bacteria  
6 used were obtained from ImmunoVet Corp. (Tampa, FL) who produced them under U.S.D.A.  
7 Product Code 9350.00. The bacteria may also be obtained from Neogen Corp. (Lansing, MI). The  
8 bacteria may be provided wet or dry. A dry form may be prepared by standard drying methods  
9 known to a person skilled in the art. such as freeze-drying or evaporation.

10 *P. acnes* may be manufactured by laboratory processes known in the art. *P. acnes* may be  
11 isolated and cultured by standard cell culture methods. The *P. acnes* product is prepared by culturing  
12 *P. acnes* on solid or in liquid media at a temperature of 36 °C +/- 2 °C for 24 to 192 hours,  
13 depending on the culture conditions. *P. acnes* may be grown on plates, e.g., agar plates containing  
14 various nutrients, or in bioreactors. The bioreactors include stationary culture flasks, shaker flasks,  
15 standard fermentors, hollow fiber reactors, perfusion reactors, plug flow reactors, etc., containing a  
16 fermentation broth with nutrients in dissolved form such as glucose, starches, tryptic soy broth,  
17 hormones, coenzymes, and optionally serum. *P. acnes* is then collected using standard separation  
18 methods such as centrifugation, and tested for purity by immunofluorescence or biochemical testing.

19 The *P. acnes* is dried while subjected to heat sufficient to inactivate and kill it. Heat-killing is  
20 preferably conducted by heating the *P. acnes* in a water bath at 74 °C to 90 °C for 60 to 90 minutes.  
21 The *P. acnes* is then weighed and suspended in a sterile saline solution at a concentration of .005 to 10  
22 mg/ml. The exact concentration is determined by the proposed use of the product, be it the treatment  
23 of warts or viral infections of the respiratory tract. The saline solution comprises sodium chloride in a  
24 buffer selected from the group consisting of alkaline metal phosphate or citrate buffers, such as sodium  
25 phosphate, potassium phosphate, sodium citrate, and potassium citrate, or sodium chloride in dI water.  
26 Preferably, the concentration of the sodium chloride is 0.85 % w/v, more preferably the concentration  
27 of the sodium chloride is 0.9 % w/v.

1            Optionally, the *P. acnes* may be mixed with carriers and fillers, and brought into the form of a  
2 therapeutically enteric pharmaceutical composition. Suitable carriers are sugars including but not limited  
3 to lactose, saccharose, mannitol, or sorbitol; cellulose preparations, amino acids such as glycine,  
4 binders such as starch pastes that use corn, wheat, rice or potato starch, gelatine, methylcellulose,  
5 hydroxypropylmethylcellulose, and sodium carboxymethylcellulose.

6            Optionally, an anesthetic may be added to the *P. acnes* product to induce local anesthesia  
7 when administered to the patient. Local anesthetics are drugs that block the generation and  
8 propagation of impulses in excitable tissues, most notably the spinal cord, spinal nerve roots, and  
9 peripheral nerves, but also skeletal muscle, cardiac muscle, and the brain. Preferably, the anesthetic is  
10 chosen from the group consisting of aminoamides, such as lidocaine (xylocaine), and aminoesters such  
11 as 2-Chloroprocaine. Preferably, the local anesthetic is lidocaine (xylocaine). Preferably, the  
12 anesthetic is added to the *P. acnes* preparation to make a final concentration of 0.25 % to 5.0 % v/v,  
13 more preferably at a final concentration of 0.5% to 2.5% v/v, and most preferably at a final  
14 concentration of 1% to 2% v/v.

15            The *P. acnes* may be lyophilized at any step in the preparation process depending on whether  
16 the final pharmaceutical formulation is to be stored as a liquid with stabilizing fillers, or as a lyophilized  
17 solid.

18            Once the *P. acnes* product is in the final vial, it is terminally sterilized by heating to 121 °C, for  
19 20 minutes, at a pressure of 15 psi.

20            The *P. acnes* product may be tested for potency using standard animal inoculation tests which  
21 consists of pre-inoculating the animal with the product, followed by a lethal challenge of a known  
22 bacterial pathogen at 1- 7 days which kills at least 75% of the non-inoculated control animals. The  
23 dosage units tested are equivalent to  $10^9$  -  $10^{13}$  *P. acnes*, preferably  $10^{10}$  -  $10^{12}$  *P. acnes*. Lidocaine  
24 (xylocaine) is added at a dosage that does not affect the potency of the formulation. The laboratory  
25 animal potency tests demonstrated that this local anesthetic does not adversely affect the potency of the  
26 product.

1 In the present invention, the autoclaved *P. acnes* product is administered intralesionally or  
2 subcutaneously to cause the regression of plantar warts in humans. The *P. acnes* product retains  
3 activity once autoclaved and once injected, and may be used with or without the addition of an  
4 anesthetic. However, the novel addition of anesthetics like lidocaine to this immune modulating  
5 preparation of *P. acnes* retains the potency of the *P. acnes* while preventing pain upon injection. The  
6 warts may be plantar, genital, or surface warts located anywhere on the skin or mucosal surface of the  
7 body. The subcutaneous route of administration of the *P. acnes* product causes a systemic reaction  
8 that causes long-term warts to completely regress. Specifically, the subcutaneous injection of the  
9 product into the arm induces the regression of warts located on the hands or feet of the patients  
10 receiving the injection. Thus, it has been determined that at doses prescribed for intralesional injections,  
11 subcutaneous injection may also be effective in causing a systemic regression of the warts. Multiple  
12 injections may be made intralesionally or subcutaneously for the purpose of treating plantar warts.  
13 Repeated doses in animals or humans have not resulted in any cumulative toxicity. Since the plantar  
14 warts are the most difficult variety of the human papilloma to treat, multiple injections may be required  
15 over time. However, a single injection may cause regression of the wart. For the regression of warts,  
16 the *P. acnes* is administered at a dose of .001 to 5 mg per dosage, preferably at a dose of .005 to 2.5  
17 mg per dosage, and more preferably at a dose of .01 to 1 mg per dosage.

18 The *P. acnes* product may also be used to treat chronic complications of the respiratory tract  
19 due to viral or bacterial infections where symptomatic coughs are persistent. The *P. acnes* product is  
20 orally administered as a treatment for acute or subacute viral infections of the respiratory tract in  
21 people, at a dose range of 0.1 to 10 mg, and more preferably at a dose range of 0.5 to 5 mg. Oral  
22 administration of the heat killed, terminally sterilized *P. acnes* saline product will hasten recovery from  
23 virally induced infections of the upper and lower respiratory tract. Optionally, an FDA approved  
24 natural or synthetic flavoring is added to the final product to make the administered product more  
25 palatable. The FDA approved natural flavorings are listed in the Code of Federal Regulations, 21 CFR  
26 172.510. The synthetic flavorings are listed in 21 CFR 172.515.



1 The complete disclosure of all patents, patent documents, and publications cited herein are  
2 incorporated by reference. The detailed descriptions and examples herein have been given for clarity of  
3 understanding only. No unnecessary limitations are to be understood therefrom. The invention is not  
4 limited to the exact details shown and described, for variations obvious to one skilled in the art will be  
5 included within the invention defined by the claims.

### 6 **Example 1**

#### 7 **Treatment of sore throat, ear ache and cough by oral administration of autoclaved, heat-killed** 8 ***P. acnes*.**

9 A sterile saline suspension of non-viable *P. acnes*, terminally autoclaved for 15 minutes at 15  
10 psi, was orally administered to patients to impede the advancing clinical signs of upper and lower  
11 respiratory tract infections, clinically manifested as sore throat, ear ache, and cough.

12 *P. acnes* was orally administered to two patients to treat the onset of symptoms of a sore throat  
13 and ear inflammation. In each case, the treatment consisted of 2 ml of a saline suspension of non-  
14 viable, heat-killed and terminally sterilized *P. acnes* at a concentration of 0.4 mg per ml. The success  
15 of the treatment demonstrates the efficacy of orally administer *P. acnes* to minimize infections of the  
16 respiratory tract in humans. Either one dose or more may be used safely to treat the symptoms of  
17 disease.

18 The first patient was a 60-year old Caucasian male weighing 190 pounds. The patient was  
19 treated with the suspension on two separate occasions. The patient had symptoms of a sore throat and  
20 ear inflammation. The treatment was administered orally. The material was held at the back of the  
21 mouth for about 1 minute before swallowing. In about 8 to 12 hours following the treatment, the patient  
22 felt somewhat flushed, a symptom that could be related to the infection or to immunostimulation. Within  
23 24 hours, the onset of the sore throat and the ear infection diminished. Within 2 days, the patient was  
24 healthy with no remaining symptoms of the sore throat and ear infection.

25 In October, 1998, the patient displayed symptoms of sneezing, coughing, nasal discharge, sore  
26 throat, and aching ears. The treatment was administered orally. The material was held at the back of

the mouth for about 1 minute before swallowing. Within the following 24 hour period, the patient again noted a slight febrile response. A second dose, similar to the first dose, was administered twenty-four hours following the first dose. No febrile response was observed after this administration. No symptoms of inflammation of the throat and ears were observed after the first day. However, mild coughing and nasal discharge continued on the second day. On the third day, the symptoms began to abate and on the fourth day, they were entirely gone.

The second patient was a 32-year old Caucasian female weighing about 140 pounds. The patient had a hoarse voice and complained of an ear ache and sore throat. She was given a similar suspension in the same amount as mentioned above. She did not express any adverse reactions or any symptoms other than those relating to her upper respiratory tract infection. The day following treatment, her throat felt better and within two days thereafter, she was again healthy.

This finding demonstrates the efficacy of orally administer *P. acnes* to minimize infections of the respiratory tract in people. Either one dose or more may be used safely to treat the symptoms of disease.

## **Example 2**

### **Preparation of *P. acnes*.**

*P. acnes*, grown on solid or in liquid media at a temperature of 36 °C for 7 days is separated, tested for purity (by immunofluorescence) and/or biochemical testing, dried while subjected to heat sufficient to kill it, weighed, and suspended in sterile saline at the desired concentration. In the final vial, the product is terminally sterilized for 20 minutes at 15 psi. Or the product can be modified by (through sterile filling) the addition of lidocaine at the desired concentration to induce local anesthesia when injected. The product is then tested for potency using the laboratory animal inoculation test which consists of pre-inoculation with the product and followed several days later by a lethal challenge of a known bacterial pathogen which kills at least 75% of the non-inoculated control animals.

## **Example 3**

**Evaluation of the safety of injecting heat-killed *P. acnes* into volunteers with plantar warts.**

1           The purpose of this Phase I Safety Study was to evaluate the safety of injecting heat-killed, *P.*  
2 *acnes* into volunteers with plantar warts. Two routes of administration were utilized, intralesional and  
3 subcutaneous. Two dose levels of experimental product (0.1 mg and 0.2 mg.) were injected. The  
4 control group was injected intralesionally with sterile saline at a volume consistent with the 0.2 mg  
5 amount of *P. acnes*. Safety parameters were assessed by changes or lack of changes in physical,  
6 hematologic, biochemical, and immunologic parameters. The lot # of the Test Article was 022497 and  
7 the Placebo was lot #KVK794220. Concentration of *P. acnes* was 0.4 mg. per milliliter. In order to  
8 test for reactions resulting in repeated injections, the volunteers received a series of three injections at  
9 intervals of one week. The patients were randomized upon entry to the study and the study was  
10 placebo controlled and blinded to the patient, but not to the investigator. The patients were monitored  
11 for four weeks following the initial injection.

12           Anticipated reactions were monitored along with changes in the blood cells, blood chemistry  
13 and in the urine. Provisions were in place to focus on any unexpected adverse reactions. The various  
14 systemic events monitored included elevated temperature, headache, muscle pain, weakness, chills,  
15 nausea, and at the injection site, pain, swelling, redness and discoloration. These are reported on each  
16 patient, grouped by treatment and recorded by severity. A summary by treatment groups of the  
17 anticipated reactions by number of patients and severity is provided. Separate summary sheets of the  
18 observed hematological, chemical and urine changes are also provided for each patient.

19           In the overall evaluation of the clinical signs designated as anticipated events, in those volunteers  
20 who designated the severity as "severe", the total events were ranked in the following order for the  
21 combined groups: elevated temperature above 100 °F. (21), pain at the injection site (15), headaches  
22 (5), chills (4), muscular pain (4), discoloration (3), weakness (2), nausea (2), swelling (2), and redness  
23 (2).

24           Where the anticipated events were designated as "moderate", the events were ranked as  
25 follows for the combined groups: temperature between 98.0 and 99.9 °F. (104), pain at the injection  
26 site (30), swelling (27), weakness (9), chills (8), headache (7), treatment groups collectively, there were  
27 8/30 complete regressions, 6/30 that were reduced in size, 10/30 that were not changed in size, 2/30

1 that were enlarged and 4/30 that were lost to follow-up. In the control group, there were no  
2 regressions, no reductions in size, 2/3 that were not changed in size and 1/3 that was enlarged.

3 These studies show that while concentrations below 0.4 mg/ml are adequate, the volumes  
4 required for efficacy are subsequently higher. Therefore, the test material should be concentrated  
5 above 0.4 mg per milliliter in order to reduce the volume of intralesional injections. Since there were a  
6 number of complete regressions in the groups where the material was administered subcutaneously, both  
7 intralesional and subcutaneous administration separately, or in combination, are efficacious.

#### 8 **Example 4.**

#### 9 **Clinical Toxicities of *P. acnes* in human subjects.**

10 *P. acnes*, manufactured within the State of Florida (ImmunoMed Corporation) has been  
11 administered intravenously to 21 cancer patients in a completed Phase I study conducted under Florida  
12 law. The patients were comprised of 14 males and 7 females, age 38 to 73 years (median = 56). The  
13 dosage per injection ranged from 25 ug to 800 ug, and the total dosage ranged from a low of 50 ug to a  
14 high of 8525 ug.

15 A total of 256 injections were administered to these patients, and 44 were associated with  
16 toxicity (17.2%). Toxicities reported included chills (24/256 - 9.4%), fever (22/256 - 8.6%), nausea  
17 (10/256 = 3.9%), myalgia (4/256 - 1.6%), malaise (2/256 - 0.8%), and lightheadedness (2/256 -  
18 0.8%). There was no injection site toxicity reported.

19 In another experiment with *P. acnes*, 3 healthy male volunteers were administered the  
20 immunostimulant I.V.. Two received 0.1 mg (0.0012 mg/kg) and the third received 0.2 mg (0.0023  
21 mg/kg.). Fever, chills, malaise, lethargy, and slight muscle soreness were experienced by all three  
22 individuals beginning 12-18 hours following injection. One individual, who received 0.2 mg, experience  
23 slight nausea without vomiting. Symptoms abated within 24 hours after onset. One individual received  
24 0.1 mg was administered a second injection of 0.1 mg 27 days after the first injection. Only a slight  
25 fever (1 F. increase) was recorded with no other symptomatology.

- 1           Intralesional and subcutaneous injections of the test material have minimally associated
- 2   toxicities. Intravenous administration should have toxicities similar to those reported previously.